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The Hmgb1 and Hmgb2 genes code for chromatin factors, which have critical roles in cellular processes, including transcription and DNA modification. In embryonic development, however, the function of Hmgb genes is largely unknown. To address this issue, we generated double mutants of Hmgb1;Hmgb2 in mice. While double null embryos arrest at E9.5, Hmgb1−/−;Hmgb2+/− embryos exhibit a loss of digit5, the most posterior digit, in the forelimb. We show that Hmgb1−/−;Hmgb2+/− forelimbs have a reduced level of Shh signaling, as well as a significant downregulation of Wnt and BMP target genes in the posterior domain. Furthermore, we demonstrate that hmgb1 and hmgb2 in zebrafish embryos enhance Wnt signaling in a variety of tissues, and that double knockdown embryos have reduced Wnt signaling and shh expression in pectoral fin buds. Our data show that Hmgb1 and Hmgb2 function redundantly to enhance Wnt signaling in embryos, and further suggest that the development of digit5 is regulated by integration of Wnt, Shh and BMP signaling in forelimbs.

doi:10.1016/j.ydbio.2011.05.405

Program/Abstract # 444
Total loss of limb bud retinoic acid signaling in Rdh10 mutants does not affect limb patterning but results in interdigital webbing

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Expression of genes controlling proximodistal (Meis2) or anteroposterior (Shh) limb patterning is purported to be controlled by retinoic acid (RA). Embryos lacking RDH10, the primary enzyme synthesizing retinaldehyde (needed for RA synthesis) during mouse development, survive until E14.5 with stunted forelimbs but apparently normal hindlimbs. Using embryos carrying the RARE-lacZ RA-reporter transgene, we show that endogenous RA activity in Rdh10 (trex/trex) mutants is detected in neuroectoderm but not limbs during initiation and patterning. Treatment of Rdh10 mutants with 25 nM RA restores RARE-lacZ activity to limb mesoderm, validating that RARE-lacZ is a sensitive marker of RA activity and verifying that RA is absent in mutant limbs. In Rdh10 mutants, hindlimbs exhibit normal Meis2/Shh expression and skeletal patterning via alcian blue staining. Forelimbs also exhibit normal Meis2 expression and only a minor alteration in Shh expression, which may be due to stunted forelimb growth, suggesting a forelimb specific initiation requirement rather than a role in patterning. Rdh10 mutants lack interdigital RA activity later in development, and accordingly fail to exhibit normal loss of interdigital mesenchyme. These findings show that Rdh10 is the sole provider of retinaldehyde needed to generate RA for the limb buds. Our analysis of Rdh10 mutants demonstrates that RA is unnecessary for control of limb patterning but required later for interdigital tissue loss.

doi:10.1016/j.ydbio.2011.05.406

Program/Abstract #445
Nucleo-cytoplasmic shuttling of Tbx5 affects migration of limb precursor cells

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Recent work from our laboratory demonstrated tbx5 transcriptional regulation by dynamic shuttling of Tbx5 between the nucleus and cytoplasm and retention of the transcription factor along the actin cytoskeleton via the PDZ-LIM protein, Pdlim7. In the zebrafish, tbx5 and pdlim7 are coexpressed in the lateral plate mesoderm, a tissue layer containing both heart and limb precursor cells. Misregulation of Tbx5 via Pdlim7 knockdown causes heart valve malformations and limb bud outgrowth problems, suggesting a basic cellular function for Tbx5/Pdlim7 interactions in the heart and limb cells. For productive limb outgrowth, Fgf signaling between the mesenchyme and the apical ectodermal ridge (AER) is essential. Blocking Pdlim7 expression prevents the switch of fgf24 from the mesenchyme to the AER, resulting in breakdown of the Fgf signaling feedback loop and precluding limb outgrowth. Prior to this signaling failure, we see protracted migration of a population of the common heart-limb field cells (hand2 positive) and reduced cell compaction at the limb fields. Knockdown of Tbx5 has been reported to cause limb cell migration defects. Considering our new findings with Pdlim7, we hypothesize that Pdlim7-mediated Tbx5 shuttling is a regulatory mechanism for cell migration. To explore this, we are currently manipulating the cellular level and subcellular location of Tbx5. To this end, we employ knockdown or overexpression of Pdlim7 in conjunction with wild type and tbx5 mutant (heartstrings) zebrafish crossed to a hand2:eGFP reporter line. Using confocal microscopy, we track the migration behavior of limb precursor cells within the living embryo as a function of Tbx5 and Pdlim7 status.

doi:10.1016/j.ydbio.2011.05.407

Program/Abstract #446
Withdrawn

doi:10.1016/j.ydbio.2011.05.408

Program/Abstract #447
Prolonged FGF signaling is necessary for foregut organ induction in *Xenopus*

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The FGF signaling pathway plays many roles in patterning the developing gut tube. During organ induction, different thresholds of FGF activity induce the lung, liver, and pancreas lineages in the mouse. The mechanism that regulates the dose of FGF in vivo is unknown. We hypothesize that prolonged FGF signaling is required and that this duration is important to help achieve the thresholds of FGF necessary for proper foregut organ induction. We tested the temporal requirement of FGF signaling by treating developing *Xenopus* embryos with soluble FGF inhibitor molecules over a time course prior to organ induction. Additionally, we injected a dominant negative FGF receptor to block FGF signaling in the endoderm. Embryos were analyzed for markers of the lung, liver, pancreas, and heart to determine if organ